

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Biochimica et Biophysica Acta 1688 (2004) 68–77



## Characterisation of 11 $\beta$ -hydroxysteroid dehydrogenases in feline kidney and liver

L. Schipper<sup>a,\*</sup>, B. Spee<sup>b</sup>, J. Rothuizen<sup>b</sup>, F. Woutersen-van Nijnanten<sup>a</sup>, J. Fink-Gremmels<sup>a</sup>

<sup>a</sup>Department of Veterinary Pharmacy, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University Utrecht, P.O. Box 80.152, 3508 TD Utrecht, The Netherlands

<sup>b</sup>Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, University Utrecht, P.O. Box 80.154, 3508 TD Utrecht, The Netherlands

Received 3 June 2003; received in revised form 27 October 2003; accepted 3 November 2003

### Abstract

11 $\beta$ -Hydroxysteroid dehydrogenases type 1 and 2 (11 $\beta$ -HSD1 and 11 $\beta$ -HSD2) are microsomal enzymes responsible for the interconversion of cortisol into the inactive form cortisone and vice versa. 11 $\beta$ -HSD1 is mainly present in the liver, and has predominantly reductase activity although its function has not yet been elucidated. 11 $\beta$ -HSD2, present in mineralocorticoid target tissues such as the kidney, converts cortisol into cortisone. Reduced activity due to inhibition or mutations of 11 $\beta$ -HSD2 leads to hypertension and hypokalemia resulting in the Apparent Mineralocorticoid Excess Syndrome (AMES). Like humans, cats are highly susceptible for hypertension. As large species differences exist with respect to the kinetic parameters ( $K_m$  and  $V_{max}$ ) and amino acid sequences of both enzymes, we determined these characteristics in the cat. Both enzyme types were found in the kidneys. 11 $\beta$ -HSD1 in the feline kidney showed bidirectional activity with predominantly dehydrogenase activity (dehydrogenase:  $K_m$  1959  $\pm$  797 nM,  $V_{max}$  766  $\pm$  88 pmol/mg\*min; reductase:  $K_m$  778  $\pm$  136 nM,  $V_{max}$  112  $\pm$  4 pmol/mg\*min). 11 $\beta$ -HSD2 represents a unidirectional dehydrogenase with a higher substrate affinity ( $K_m$  184  $\pm$  24 nM,  $V_{max}$  74  $\pm$  3 pmol/mg\*min). In the liver, only 11 $\beta$ -HSD1 is detected exerting reductase activity ( $K_m$  10462 nM,  $V_{max}$  840 pmol/mg\*min). Sequence analysis of conserved parts of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 revealed the highest homology of the feline enzymes with the correspondent enzymes found in man. This suggests that the cat may serve as a suitable model species for studies directed to the pathogenesis and treatment of human diseases like AMES and hypertension.

© 2003 Elsevier B.V. All rights reserved.

**Keywords:** 11 $\beta$ -Hydroxysteroid dehydrogenase; Enzyme kinetics; Sequence; Cat; Apparent mineralocorticoid excess syndrome; Hypertension

### 1. Introduction

The microsomal 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ -HSDs) convert bioactive glucocorticosteroids (cortisol and corticosterone) into their inactive keto-metabolites (cortisone and dehydrocorticosterone) and vice versa. Therefore, 11 $\beta$ -HSDs play an important role in the regulation of local levels of bioactive glucocorticoids, resulting in different physiological effects. Two distinct types of 11 $\beta$ -HSD have been characterised extensively. They not only differ in kinetic characteristics, such as prevailing reaction direction, Michaelis–Menten constant ( $K_m$ ), maximum enzyme velocity ( $V_{max}$ ) and co-substrate

dependency, but also in molecular weight and amino acid sequence [1].

The first described type (11 $\beta$ -HSD1) is mainly present in the liver but also found in many other tissues [2,3] and although this enzyme is bidirectional, reductase activity dominates. This enzyme requires NADP(H) as cofactor [4–9]. Another feature is the relatively low substrate affinity (Michaelis–Menten constant in the micromolar range) in comparison to 11 $\beta$ -HSD2. The molecular weight of 11 $\beta$ -HSD1 is approximately 34 kDa [2]. The cofactor binding pocket and the active site are highly conserved sites of the enzyme [10]. The exact function of 11 $\beta$ -HSD1 is not yet fully elucidated; besides the conversion of glucocorticosteroids, this enzyme might also play a role in detoxification processes [11], inflammation [12], insulin sensitivity [13] and obesity [14].

In contrast to 11 $\beta$ -HSD1, 11 $\beta$ -HSD2 is a unidirectional dehydrogenase as it converts the biologically active glu-

\* Corresponding author. Tel.: +31-253-53-27; fax: +31-253-41-27.

E-mail address: [L.Schipper@vfft.vet.uu.nl](mailto:L.Schipper@vfft.vet.uu.nl) (L. Schipper).

cocorticosteroid cortisol into its biologically inactive form cortisone. For this conversion, NAD is required [15]. The  $K_m$  is in the nanomolar range, indicating a higher substrate affinity than  $11\beta$ -HSD1. The molecular weight is approximately 44 kDa [16,17] although also a molecular weight is described of 41 kDa [18,19] and 48 kDa [17]. The enzyme consists of five conserved regions (A–E), with the cofactor binding site (A) and the active site (D) as the most important [20]. Sequence analysis of human  $11\beta$ -HSD2 showed only 18% homology with human  $11\beta$ -HSD1.  $11\beta$ -HSD2 is mainly present in mineralocorticoid target tissues such as the kidney, salivary gland and distal colon, but has also been found in the placenta. In the kidney, it co-localises with the mineralocorticoid receptor, which has equal affinity for aldosterone and the bioactive glucocorticoid cortisol. In this way,  $11\beta$ -HSD2 preserves aldosterone specificity on the nonselective mineralocorticoid receptor by inactivating glucocorticoids locally [21]. Partial or complete inhibition of  $11\beta$ -HSD2 activity in mineralocorticoid target tissues results in hypertension and hypokalemia due to overstimulation of the mineralocorticoid receptor by cortisol which circulates in much higher levels than aldosterone [22]. In the placenta,  $11\beta$ -HSD2 protects the foetus against high circulating levels of maternal glucocorticoids [23]. Decreased activity in the placenta is associated with low birth weight and development of hypertension later in life [24]. In humans, several point mutations in the gene encoding for  $11\beta$ -HSD2 are described. Each of these is responsible for mild to severe abnormalities in cortisol metabolism and hypertension [25]. Moreover, experimental gene deletion of  $11\beta$ -HSD2 in mice resulted in severe hypertension in this species [26].

It is well known that remarkable species differences exist with respect to tissue distribution of the  $11\beta$ -HSD types: in human kidney predominantly  $11\beta$ -HSD2 is present [19,20,27], while in the kidney of the rat [28–31], guinea pig [5,6] and mouse [32,33]  $11\beta$ -HSD1 and  $11\beta$ -HSD2 have been demonstrated by both activity studies and RT-PCR.  $11\beta$ -HSD1 is present in liver tissue of rat [7,28,29,34], mouse [32] [35], man [27,36] and guinea pig [5,6], while in koala liver this enzyme is absent [37].

Another feature that differs between the species is the predominant activity of  $11\beta$ -HSD1. In guinea pig [6], mouse [35], sheep and human liver microsomes [27],  $11\beta$ -HSD1 has bidirectional activity, but reductase activity prevails. In rats, large interstrain differences have been found. In Wistar–Kyoto rats bidirectional activity was established [28] whereas in Sprague–Dawley rats [7] only dehydrogenase activity has been detected.

The enzyme kinetics of  $11\beta$ -HSD2 show more constancy in the different species investigated. The main activity of this NAD-dependent enzyme is dehydrogenation, and the substrate affinity is higher than the substrate affinity of  $11\beta$ -HSD1.

Since  $11\beta$ -HSD2 is known as the NAD-dependent form of  $11\beta$ -HSD, controversial findings have been reported in mouse [35], rat [28] and guinea pig [5,6], as besides the NADP(H)-dependent form ( $11\beta$ -HSD1), also a bidirectional NAD(H)-dependent enzyme has been found in the liver. As even Northern blot analysis did not confirm the presence of  $11\beta$ -HSD2 in the liver of these species [5,33,38], it was suggested that other forms of  $11\beta$ -HSD might exist.

In contrast to other animal species, cats are susceptible for hypertension as known from clinical studies [39]. However, in hypertension research, most often rats [40,41] and mice [42] are used. These animals have corticosterone as physiological glucocorticosteroid, while humans and cats have cortisol. Although both glucocorticosteroids are converted by  $11\beta$ -HSD, the affinity of the glucocorticosteroids for the enzyme differs considerably [35,43].

As impaired cortisol metabolism, due to inhibition or mutation of  $11\beta$ -HSD2, plays a role in the pathogenesis of hypertension in man and probably explains the high susceptibility of cats for hypertension, we characterised both  $11\beta$ -HSDs by assessing enzyme kinetic parameters in feline kidney- and liver microsome fractions. For confirmation of distinct  $11\beta$ -HSD1 and  $11\beta$ -HSD2 enzymes, cDNA derived from cat liver and kidney was sequenced.

## 2. Materials and methods

[1,2,6,7- $^3$ H] cortisol (60.0 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Arlington Height, IL, USA) and [1,2- $^3$ H] cortisone (50 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA). Nonradioactive steroids and the cofactors (NAD, NADH, NADP, NADPH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). TLC-plates (silica gel 60 F<sub>254</sub>, 20 × 20 cm) and ethyl acetate were from Merck (Darmstadt, Germany). The scintillation fluid was Ultima Gold from Packard Bioscience (USA). All other chemicals were of analytical grade. Fresh kidney and liver tissue were obtained from three clinical healthy male cats (*Felis catus*). No abnormalities were found neither during serum analysis (urea, creatinine, bile acids, ALAT, calcium, total protein, albumin) nor in hematocrit values and leukocyte counts.

For sequence analyses, RNA of both tissues was isolated using Qiagen RNeasy Mini Kit. The Dnase was purchased from Qiagen Rnase-free Dnase kit. The poly(dT) primers were incubated in the Reverse Transcription System from Promega (Leiden, the Netherlands). Platinum *Taq* polymerase was purchased from Invitrogen (The Netherlands). To purify the PCR product, QIAquick Gel Extraction Kit (Qiagen) was used. The sequencing was performed with ABI PRISM Big Dye Terminator Cycle Sequencing Kit. The fragments were analysed with an automatic sequencer ABI PRISM model 3100 (Applied Biosystems) (The Netherlands).

### 2.1. Isolation of feline liver and kidney microsomes

After removal of the renal capsule and the pelvis, the kidneys were put in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until further preparation. From the livers, representative cross sections were taken and stored in the same way. Microsomes were prepared by a standard procedure as described previously [44]. In short, all tissues were homogenised with a Potter S (B. Braun Biotech International, Melsungen, Germany) in 2 volumes of ice-cold potassium chloride (1.15%) containing 0.1 mM EDTA. All subsequent steps were performed at  $0-4^{\circ}\text{C}$ . The tissue homogenate was centrifuged at  $10,000 \times g$  for 25 min. The supernatant was decanted and centrifuged at  $100,000 \times g$  for 75 min. The pellet was suspended in KCl/EDTA solution, homogenised with a Ultra-turrax T25 (Janke and Kunkel, Germany) and again centrifuged at  $100,000 \times g$  for 75 min. Finally the pellet was homogenised again with the Ultra-turrax and resuspended in phosphate buffer (pH 7.4, 1.5 mM  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.1 mM EDTA) containing 20% glycerol, frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until use. Protein quantification was performed with the method described by Lowry [45].

### 2.2. Assay of 11 $\beta$ -dehydrogenase activity

Liver and kidney microsomes were pooled from three male cats. Incubations were carried out in 2-ml Eppendorf micro-test tubes in a  $37^{\circ}\text{C}$  water bath for 15 min. The total incubation volume was 250  $\mu\text{l}$ , containing 4 mM cofactor ( $\text{NAD}^+$ ,  $\text{NADP}^+$ ), [ $^3\text{H}$ ]-cortisol (0.5  $\mu\text{Ci}$  per incubation) and unlabeled cortisol (30 nM–1500 nM in the  $\text{NAD}$ -dependent experiments, 420–25,000 nM in the  $\text{NADP}$ -dependent experiments) and 0.05M phosphate buffer (pH 7.4). The reaction was started by adding the microsome fraction. After 15 min, the reaction was arrested by addition of 1.5 ml ethyl acetate and putting the samples on ice. The ethyl acetate fraction was transferred to a tube containing 50  $\mu\text{l}$  (1.1 mM) unlabeled cortisol and 50  $\mu\text{l}$  (1.1 mM) cortisone to mark the position of [ $^3\text{H}$ ]-labelled steroids on TLC plates later on. After evaporation of the ethyl acetate under nitrogen, the residue was dissolved in chloroform/methanol (50:50 v/v) and separated by TLC using chloroform/methanol (9:1, v/v) as a mobile phase. The spots containing cortisol and cortisone were detected by UV illumination, cut out and transferred into separate vials containing 12 ml scintillation liquid. To elute the labelled steroids from the TLC matrix, the vials were shaken for 2 h. To avoid loss of accuracy by air bubbles, the radioactivity was measured 48 h later using a 4700 PW liquid scintillation counter. In initial studies, the protein concentration in each reaction was adjusted to ensure the linearity of product formation over the incubation time. To keep the conversion rates between 5% and 40%, the amount of

protein per incubation was optimised for every cofactor-dependent experiment (0.020 and 0.016 mg in the experiments in the kidney with  $\text{NAD}$  and  $\text{NADP}$ , respectively). All values were corrected with blank incubations, in which all assay components were present, except the microsomes.

### 2.3. Assay of 11-reductase activity

11-Reductase activity was determined similar to the 11 $\beta$ -dehydrogenase assay except that  $^3\text{H}$ -cortisone (0.5  $\mu\text{Ci}$  per incubation) and cortisone (420–25000 nM) were used as substrates and  $\text{NADH}/\text{NADPH}$  as cofactors. Optimised conditions for the kidney experiments were 0.041 mg protein and an incubation time of 15 min, and in the liver 0.058 mg protein and 30 min incubation time.

### 2.4. Analysis of enzyme kinetic data

The enzyme velocity was determined by calculating of the conversion rate of cortisol into cortisone or the backward conversion. Data are expressed in picomoles per milligram of protein per minute (pmol/mg min). All data reported represent means of three to five independent incubations. Kinetic analyses were performed with EZ-FIT [46]. Significance of the effects of cofactors on  $K_m$  and  $V_{\max}$  was evaluated using a two-sided unpaired Student's  $t$  test.

### 2.5. RT-PCR

Total cellular RNA was isolated from frozen feline tissues by using Qiagen RNeasy Mini Kit according to the manufacturer's instructions. Total RNA (1  $\mu\text{g}$ ) was incubated with poly(dT) primers using the Reverse Transcription System at  $42^{\circ}\text{C}$  for 15 min, followed by inactivation at  $95^{\circ}\text{C}$  for 5 min. Putative feline 11 $\beta$ -HSD1 primers (set 11 $\beta$ -HSD1: sense, 5'-TTCAGACCAGAGATGCTCC-3'; antisense, 5'-CCTTTGATGATCTC-CAGGGC-3'), were designed from the conserved regions of human (AY044083), sheep (AF414124), and rat 11 $\beta$ -HSD1 (NM\_0127080) cDNAs. Putative feline 11 $\beta$ -HSD2 primers (set 11 $\beta$ -HSD2: sense, 5'-CCGGCTGTGACTCTGGTTTTG-3'; antisense, 5'-CCATGCAAGTGCTCGATGTAGTC-3') were designed from the conserved regions of human (U27317), sheep (AF414125), and rat 11 $\beta$ -HSD2 (NM\_017081) cDNAs. The PCR reaction mixture contained 2.5 units of Platinum Taq polymerase, 20 pmol each of sense and anti-sense primer, 200  $\mu\text{M}$  dNTPs, 50 mM KCl, 15 mM Tris-HCl, and 0.5 mM  $\text{MgCl}_2$ . cDNA was amplified for 40 cycles under the following conditions: denaturation at  $94^{\circ}\text{C}$  (1 min), annealing at  $50^{\circ}\text{C}$  for 11 $\beta$ -HSD1 and at  $55^{\circ}\text{C}$  for 11 $\beta$ -HSD2 (1 min), and extension at  $72^{\circ}\text{C}$  (1 min). PCR products were separated by electrophoresis on an ethidium bromide-stained agarose gel (1.5%) using

0.5 × Tris–borate EDTA buffer and were visualised on an ultraviolet transilluminator. The PCR products (11 $\beta$ -HSD1: 664 bp and 11 $\beta$ -HSD2: 652 bp) were cut out of the agarose-gel and purified using the QIAquick Gel Extraction Kit.

Sequences of both strands were obtained using Standard sequencing procedures (ABI PRISM 3100 Genetic Analyser, Applied Biosystems, Foster City, CA, USA).

### 3. Results

#### 3.1. Kinetic parameters of 11 $\beta$ -HSD in feline kidney

In feline kidney a high affinity unidirectional NAD-dependent dehydrogenase was found. These characteristics are typical for 11 $\beta$ -HSD2. Moreover, a bidirectional NADP(H)-dependent enzyme was found, with equal low affinity for cortisol and cortisone, but with a higher  $V_{\max}$  for dehydrogenation of cortisol into cortisone ( $766 \pm 88$  pmol/mg min) than for the reduction of cortisone into cortisol ( $112 \pm 4$  pmol/mg\*min). The conversion of cortisol into cortisone and the reverse reaction follow Michaelis–Menten kinetics as the Lineweaver–Burk plot shows linearity (for example see Fig. 1). The enzyme

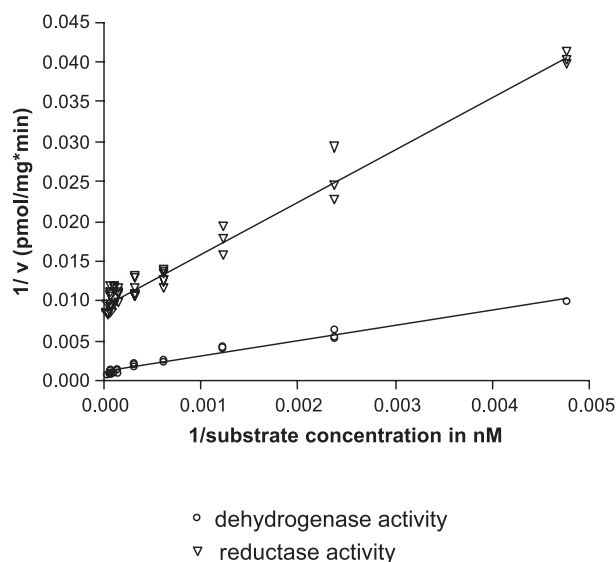


Fig. 1. Lineweaver–Burk plot of NADP-dependent dehydrogenase and NADPH-dependent reductase in feline kidney microsomes. The microsomes were incubated with  $^3\text{H}$ -cortisol and unlabelled cortisol in the presence of excess of NADP at 37 °C for 15 min for the NADP dehydrogenase activity. For the reductase activity, the microsomes were incubated with  $^3\text{H}$ -cortisone and unlabelled cortisone in the presence of excess of NADPH at 37 °C for 30 min. The  $K_m$  for the dehydrogenase reaction is  $1959 \pm 797$  nM and the apparent  $V_{\max}$  is  $766 \pm 88$  pmol/mg\*min (mean of 3 experiments  $\pm$  S.E.). The  $K_m$  for the reductase reaction is  $778 \pm 136$  nM and the apparent  $V_{\max}$  is  $112 \pm 4$  pmol/mg\*min (mean of 3 experiments  $\pm$  S.E.).

Table 1

Kinetic parameters of feline kidney 11 $\beta$ -HSD

Kidney	Dehydrogenase activity		Reductase activity	
	Cortisol $\rightarrow$ Cortisone		Cortisone $\rightarrow$ Cortisol	
Conversion				
Cofactor	NAD	NADP	NADH	NADPH
$K_m$ (nM)	$184 \pm 24$	$1959 \pm 797$	n.d.	$778 \pm 136$
$V_{\max}$ (pmol/mg min)	$74 \pm 3$	$766 \pm 88$	n.d.	$112 \pm 4$

Enzyme kinetic parameters of 11 $\beta$ -HSD in the feline kidney. Dehydrogenation of cortisol and reduction of cortisone with NAD(H)/NADP(H) are determined. Values are means  $\pm$  S.E. of three experiments. n.d. means not detectable.

kinetics of the conversion of cortisol and cortisone are listed in Table 1.

#### 3.2. Kinetic parameters of 11 $\beta$ -HSD in feline liver

Liver microsomes of cat showed unidirectional NADPH-dependent reductase activity. No dehydrogenation could be detected. The mean  $K_m$  of this reductase reaction was 10462 nM and the mean  $V_{\max}$  was 182 pmol/mg min. The NADPH-regenerating system did not elevate the reaction velocity (data not shown). Enzyme kinetics of 11 $\beta$ -HSD in the liver are summarised in Table 2.

#### 3.3. RT-PCR of 11 $\beta$ -HSD1

The primers for 11 $\beta$ -HSD1 were based on conserved regions of 11 $\beta$ -HSD1 in three species (human, sheep, rat) with the primers as described in Materials and methods. In feline liver and kidney a 581-bp fragment was detected (see Fig. 2).

The sequence of the fragment found with 11 $\beta$ -HSD1 primers in the liver and the kidney was: AAGTGGATTGT-CACAGGGGCCAGCAAGGGGATTGGAGAACA-GATGGCCTATCATCTGGCAAAGATGGGAGCC-CATGTGGTGGTGACAGCAAGGTCTAAAGAAAATC-TAAAGAAGATAGTATCCCATTGCATGGAGCTTG-GAGCAGCCTCAGCACACTACATTGCTGGCACCATG-GAGAATATGACCTTCGCAGAGCAATTTGTTGC-CAAAGCTGGAAAGCTCATGGGGGGGACTAGA-CATGCTTATTCTCAACCACATCACCAACACTTCTAT-

Table 2

Kinetic parameters of feline liver 11 $\beta$ -HSD

Liver	Dehydrogenase activity		Reductase activity	
	Cortisol $\rightarrow$ Cortisone		Cortisone $\rightarrow$ Cortisol	
Conversion				
Cofactor	NAD	NADP	NADH	NADPH
$K_m$ (nM)	n.d.	n.d.	n.d.	$10,462 \pm 840$
$V_{\max}$ (pmol/mg min)	n.d.	n.d.	n.d.	$182 \pm 6$

Enzyme kinetic parameters of 11 $\beta$ -HSD in the feline liver. Dehydrogenation of cortisol and reduction of cortisone with NAD(H)/NADP(H) are determined. Values are means  $\pm$  S.E. of three experiments. n.d. means not detectable.



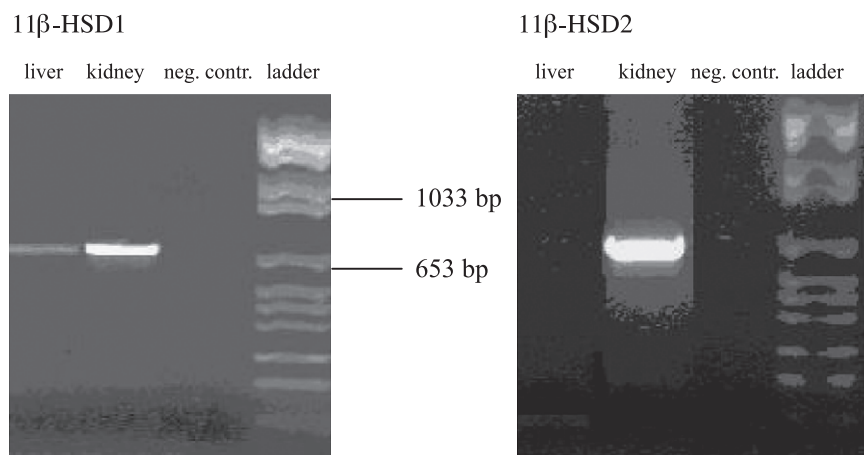


Fig. 2. Fragments of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 in the liver and kidney of the cat. The primers for 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 were based on conserved regions of the enzymes in three species (human, sheep, rat). In the kidney both 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 are found, while in the liver only 11 $\beta$ -HSD1 could be detected.

GAATCTGTTTAGTGGTGATATCCACATCGTGCGCA-GAAGCATGGAAGTCAACTTCCTCAGTTATGTGGTC-CTGAGTGCTACTGCCTTGCCTATGCTGAAGCAGAG-CAATGGAAGCATTGTTGTCTGTCCTC-GAAGGCTGGGAAAATGGCTAGTCCACT-TATTGCGCCCTATTCTGCAAGCAAGTTTGCCCTG-GATGGGTTTTTCTCCTCCATCAGGATGGAACATT-CAGTGGCCAAGATTAATGTATCGATCACTCTCTG-TATCCTCGGCCTCATAAACACAGACACAGCCAT-GAATGCCATTCTGGA.

The amino acid sequence of this RT-PCR fragment is homologous to 11 $\beta$ -HSD1 in various species. Highest homology (83,4%) was found with 11 $\beta$ -HSD1 from humans (*Homo sapiens*, see Table 3). This confirms the presence of 11 $\beta$ -HSD1 in the feline liver and kidney. Details on the homology of feline 11 $\beta$ -HSD1 with other species are listed in Fig. 3.

The cofactor binding pocket of 11 $\beta$ -HSD1 in the cat has a unique sequence, which differs in two amino acids from the other species (amino acids 48 and 49). The active site of 11 $\beta$ -HSD1 (amino acids 183–200) shows highly conserved

parts. Amino acid 199 varies between the species, including the cat. Amino acid 197 is isoleucine in man, mouse, rat and cat, whereas in sheep, cow and guinea pig, leucine is at position 197.

### 3.4. RT-PCR of 11 $\beta$ -HSD2

The primers for 11 $\beta$ -HSD2 were based on conserved regions of 11 $\beta$ -HSD2 in three species (human, sheep, rat) and tested in feline kidney and liver. Only in the kidney, a corresponding fragment was found (see Fig. 2). The sequence of this 666-bp fragment was: TTTTGCGCCCTCCGCGC-CTTTCGGTGGCCACTCGCGCGGTGCTCAT-CACCGGCTGTGACTCTGGTTTTGGCAAGGA-GACGGCAAAGAAGCTAGACGCTATGGGCTT-CACGGTGCTGGCCACCGTGTTGGAGTTGGATGGC-CCTGGTGCCCTAGAGCTGCGTGCCCTGCTGTTCCCC-TGCGCTAAGGCTGCTGCAGCTGGACCTGACCAAG-CCAGCAGACATCAGCCGAGCACTGGAGTTCACAA-AGGCCACACACCAGCAGAGGCTTGTGG-GGCCTGGTCAACAATGCAGGCCACAACAATG-TAGTGGCCGATGTGGAGCTGTCTCCAGTGGCCAC-ATTCCGCAGCTGCATGGAGGTGAATTTCTTCGGT-GCGCTTGAACTAACCAGGGCCTGTTGC-CACTGTGCGGCGTTCTAGGGGTGCGATCGT-GACTGTGGGCAGCCGAGCAGGAGACATGCCA-TACCCATGCTTGGCGGCCTATGGGTCCTC-CAAAGCGGCCATGGCACTGCTCATGGACACATT-CAGCTGTGAACCTTCTGCCCTGGGGGGTCAAGGT-CAGCATCATCCAGCCTGGCTGCTTCAAGACA-GAGTCTGTGCAGAATGTGGGCCAGTGGGAG-CAGCGTAAACAGCTGCTGCTGGCCAGCCTGCCCC-GAGAGCTGCTCCAG.

The amino acid sequence of this RT-PCR fragment is homologous to 11 $\beta$ -HSD2 in various species. The highest homology (89,6%) was found with humans (Table 4).

Table 3

Homology between feline 11 $\beta$ -HSD1 and other species, based on a 192-amino-acid fragment

		Percent identity								
Divergence		1	2	3	4	5	6	7		
	1	83.4	78.8	76.7	78.2	76.1	73.1		1	<i>Felis catus</i>
	2	18.8	77.7	76.3	78.1	79.3	73.3		2	<i>Homo sapiens</i>
	3	25.0	26.5	86.4	75.0	77.2	72.9		3	<i>Mus musculus</i>
	4	28.0	28.5	15.0	72.5	76.1	72.8		4	<i>Rattus norvegicus</i>
	5	25.8	26.0	30.4	34.3	96.2	72.3		5	<i>Ovis aries</i>
	6	24.6	24.2	27.3	28.8	3.9	72.3		6	<i>Bos taurus</i>
	7	33.4	33.0	33.6	33.8	34.6	34.6		7	<i>Cavia porcellus</i>
		1	2	3	4	5	6	7		

M A F M K K Y L L P I L G L F L A Y Y Y S A N E E F R P E M L Q G K K V I V T G A S K G I G R E M Majority																															
10					20					30					40					50											
1																					- - - V I V I T G A S K G I G E O M										Felis Cattus HSD-1
1	M A F M K K Y L L P I L G L F M A Y Y Y Y S A N E E F R P E M L Q G K K V I V T G A S K G I G R E M																														Homo Sapiens HSD-1
1	M A V M K N Y L L P I L V L S L A Y Y Y Y S T N E E F R P E M L Q G K K V I V T G A S K G I G R E M																														Mus Musculus HSD-1
1	- - - M K K Y L L P V L V L C L G - Y Y Y S T N E E F R P E M L Q G K K V I V T G A S K G I G R E M																														Rattus Norvegicus HSD-1
1	M A F M K K Y L L P I L G I F L A Y Y Y Y S A N E E F R P E M L R G K R V I V T G A S K G I G R E M																														Ovis Aries HSD-1
1											- - - E M L R G K R V I V T G A S K G I G R E M																				Bos Taurus HSD-1
1	M A F L K K Y L L T I L M V F L A Y Y Y Y P A N E K F R P E M L Q G K K V I V T G A S K G I G R E I																														Cavia Porcellus HSD-1
A Y H L A K M G A H V V V T A R S E E G L Q K V V S R C L E L G A A S A H Y I A G T M E D M T F A E Majority																															
60					70					80					90					100											
15	A Y H L A K M G A H V V V T A R S K E N L K K I V S H C M E L G A A S A H Y I A G T M E N M T F A E																														Felis Cattus HSD-1
51	A Y H L A K M G A H V V V T A R S K E T L Q K V V S H C L E L G A A S A H Y I A G T M E D M T F A E																														Homo Sapiens HSD-1
51	A Y H L S K M G A H V V L T A R S E E G L Q K V V S R C L E L G A A S A H Y I A G T M E D M T F A E																														Mus Musculus HSD-1
47	A Y H L S K M G A H V V L T A R S E E G L Q K V V S R C L E L G A A S A H Y I A G T M E D M A F A E																														Rattus Norvegicus HSD-1
51	A Y H L A R M G A H V V V T A R S E E S L K K V V S R C L E L G A A S A H Y V A G T M E N M T F A E																														Ovis Aries HSD-1
22	A Y H L A R M G A H V V V T A R S E E S L K K V V S R C L E L G A A S A H Y V A G T M E N M T F A E																														Bos Taurus HSD-1
51	A Y H L A K M G A H V V V T A R S K E A L Q K V V A R C L E L G A A S A H Y I A G S M E D M T F A E																														Cavia Porcellus HSD-1
Q F V A K A G K L M G G L D M L I L N H I T Y T S L S L F H D D I H L V R R S M E V N F L S Y V V L Majority																															
110					120					130					140					150											
65	Q F V A K A G K L M G G L D M L I L N H I T N T S M N L F S G D I H I V R R S M E V N F L S Y V V L																														Felis Cattus HSD-1
101	Q F V A Q A G K L M G G L D M L I L N H I T N T S L N L F H D D I H H V R K S M E V N F L S Y V V L																														Homo Sapiens HSD-1
101	Q F I V K A G K L M G G L D M L I L N H I T Q T S L S L F H D D I H S V R R V M E V N F L S Y V V M																														Mus Musculus HSD-1
97	R F V V E A G K L L G G L D M L I L N H I T Q T T M S L F H D D I H S V R R S M E V N F L S Y V V L																														Rattus Norvegicus HSD-1
101	Q F V A K A G E L V G G L D M L I L N H I N Y T P L R V F S N D I H L L R R S L E V N L L S Y V V L																														Ovis Aries HSD-1
72	Q F V A K A G E L V G G L D M L I L N H I H Y T P L G V F S N D I H L L R R T L E V N L L S Y V V L																														Bos Taurus HSD-1
101	E F A A E A G N L M G G L D M L I L N H V L Y N R L T F F H G E I D N V R K S M E V N F H S F V V L																														Cavia Porcellus HSD-1
S T A A L P M L K Q S N G S I V V V S S V A G K I A Q P L I A A Y S A S K F A L D G F F S S I R T E Majority																															
160					170					180					190					200											
115	S A T A L P M L K Q S N G S I V V V S S K A G K M A S P L I A P Y S A S K F A L D G F F S S I R M E																														Felis Cattus HSD-1
151	T V A A L P M L K Q S N G S I V V V S S L A G K V A Y P M V A A Y S A S K F A L D G F F S S I R K E																														Homo Sapiens HSD-1
151	S T A A L P M L K Q S N G S I A V I S S L A G K M T Q P M I A P Y S A S K F A L D G F F S T I R T E																														Mus Musculus HSD-1
147	S T A A L P M L K Q S N G S I A I I S S M A G K M T Q P L I A S Y S A S K F A L D G F F S T I R K E																														Rattus Norvegicus HSD-1
151	S T A A L P M L K Q T S G S I V V V S S V A G K I A C P L A A A Y S A S K F A L D G F F S S L R T E																														Ovis Aries HSD-1
122	S T A A L P M L K Q T N G S I V V V S S I A G K I A C P L A A A Y S A S K F A L D G F F S S L R M E																														Bos Taurus HSD-1
151	S V A A M P M L M Q S Q G S I A V V S S V A G K I T Y P L I A P Y S A S K F A L D G F F S T L R S E																														Cavia Porcellus HSD-1
Y L V T K V N V S I T L C V L G L I D T E T A M K A V S G I I L A Q A S P K E E C A L E I I K G T A Majority																															
210					220					230					240					250											
165	H S V A K I N V S I T L C I L G L I N T D T A M N A I S G																														Felis Cattus HSD-1
201	Y S V S R V N V S I T L C V L G L I D T E T A M K A V S G I V H M Q A A P K E E C A L E I I K G G A																														Homo Sapiens HSD-1
201	L Y I T K V N V S I T L C V L G L I D T E T A M K E I S G I I D A Q A S P K E E C A L E I I K G T A																														Mus Musculus HSD-1
197	H L M T K V N V S I T L C V L G F I D T E T A L K E T S G I I L S Q A A P K Q E C A L E - I K G T V																														Rattus Norvegicus HSD-1
201	Y E A T K V N V S I T L C I L G L I D T D T A M K A V A G I Y N A E A S P K E E C A L E I I K G G A																														Ovis Aries HSD-1
172	Y E A T K V N V S I T L C																														Bos Taurus HSD-1
201	F L V N K V N V S I T L C I L G L I D T E T A I K A T S G I Y L G P A S P K E E C A L E I I K G T A																														Cavia Porcellus HSD-1
L R Q D E V Y Y D K S - W T P L L L G N P G R K I M E F L S L R S Y N M D R F V S N - - - - - Majority																															
260					270					280					290					300											
193																															Felis Cattus HSD-1
251	L R Q E E V Y Y D S S L W T T L L I R N P C R K I L E F L Y S T S Y N M D R F I N K																														Homo Sapiens HSD-1
251	L R K S E V Y Y D K S L P L T P I L L G N P G R K I M E F F S L R Y Y N K D M F V S N																														Mus Musculus HSD-1
246	L R K D E V Y Y D K S S W T P L L L G N P G R R I M E F L S L R S Y N R D L F V S N																														Rattus Norvegicus HSD-1
251	L R Q D E V Y Y D N S I L T S L L L K N P G R K I M E F L S L K K Y N M E R F I N N																														Ovis Aries HSD-1
184																															Bos Taurus HSD-1
251	L R Q D E M Y Y V G S R W V P Y L L G N P G R K I M E F L S A A E Y N W D N V L S N E K L Y G R W A																														Cavia Porcellus HSD-1

Fig. 3. Amino acid sequence of a part of feline 11 $\beta$ -HSD1 in comparison with other species. The underlined amino acids indicate the cofactor binding pocket (34–63), highly conserved consensus sites for *N*-glycosylation (162 and 217), and the active site of the enzyme (183–200).

Table 4

Homology between feline 11 $\beta$ -HSD2 and other species, based on a 220-amino-acid fragment

Percent identity							
Divergence	1	2	3	4	5	6	
	1	89.6	82.4	82.0	84.7	87.4	1 <i>Felis cattus</i>
	2	11.2	80.3	83.4	86.5	86.5	2 <i>Homo sapiens</i>
	3	20.1	22.5	77.7	80.3	81.1	3 <i>Cavia porcellus</i>
	4	20.7	18.8	25.8	81.1	80.8	4 <i>Bos taurus</i>
	5	17.2	14.3	20.1	21.5	94.8	5 <i>Mus musculus</i>
	6	13.8	15.3	21.5	21.8	5.1	6 <i>Rattus norvegicus</i>
		1	2	3	4	5	6

Details on the homology of feline 11 $\beta$ -HSD2 with other species are listed in Fig. 4.

#### 4. Discussion

In the kidney of the cat, the presence of two 11 $\beta$ -HSD types could be established. One of the enzymes was a unidirectional NAD-dependent dehydrogenase with a  $K_m$  in the nanomolar range. These characteristics are indicative for 11 $\beta$ -HSD2 [15]. The other type found in the kidney

MERWFWPSGGAWLLVAARALLQLLRSDLRRLGRPLLAALLAALDWL CQR										Majority
1	---	---	---	---	---	---	---	---	---	Felis Catus HSD-2
1	MERWFWPSGGAWLLVAARALLQLLRSDLRRLGRPLLAALLAALDWL CQR									Homo Sapiens HSD-2
1	---	---	---	---	---	---	---	---	---	Cavia Porcellus HSD-2
1	MESWFWPSGGAWLLVPAARALLQLLRADLRRLGRPLLAALLAALDWL CQR									Bos Taurus HSD-2
1	MERWFWPSGGAWLLVAARALLQLLRSDLRRLGRPLLAALLAALDWL CQR									Mus Musculus HSD-2
1	MERWFWPSGGAWLLVAARALLQLLRSDLRRLGRPLLAALLAALDWL CQR									Rattus Norvegicus HSD-2
LLPPPAALAVLAAAGWIALSRLARPPRLPVATRAVLITGCD SGFGKETAK										Majority
1	---	---	---	---	---	---	---	---	---	Felis Catus HSD-2
51	LLPPPAALAVLAAAGWIALSRLARPPRLPVATRAVLITGCD SGFGKETAK									Homo Sapiens HSD-2
6	LLPPVVALAVLAATGWITLSRLARPPRLPVATRAVLITGCD TGF									Cavia Porcellus HSD-2
51	LLPPLAALAVLAATGWIVLSRLARPPRLPVATRAVLITGCD S									Bos Taurus HSD-2
51	LLPPPAALVVLAVLAGAGWIALSRLARPPRLPVATRAVLITGCD T									Mus Musculus HSD-2
51	LLPPPAALVVLAVLAGAGWIALSRLARPPRLPVATRAVLITGCD T									Rattus Norvegicus HSD-2
KLDAMGFTVLA TVLDLNSPGAELRLACCS PRLKLLQMDLT KPEDISR VLE										Majority
30	KLDAMGFTVLA TVLELDGPGAELRLACCS PRLRLLQLDLTKPADISR ALE									Felis Catus HSD-2
101	KLD SMGFTVLA TVLELNSPGAELRLACCS PRLRLLQLMDLT KPGDISR VLE									Homo Sapiens HSD-2
56	KLDAMGFMVLA TVLDLDSPGAELRLACCS SRLKLLQMDLMKPEDISR VLE									Cavia Porcellus HSD-2
101	KLD TMGFTVLA TVLDLNSPGAELRLACCS SRLKLLQMDLT KPGDISR VLE									Bos Taurus HSD-2
101	KLDAMGFTVLA TVLDLNSPGAELRLACCS PRLKLLQMDLT KPAEDISR VLE									Mus Musculus HSD-2
101	KLDAMGFTVLA TVLDLNGPGAELRLACCS PRLKLLQMDLT KPEDISR VLE									Rattus Norvegicus HSD-2
FTKAHTTSTGLWGLVNNAGLNIVVADVELSPVATFRSCMEVNFFFGALELT										Majority
80	FTKAHTTSTGLWGLVNNAGHNNVADVELSPVATFRSCMEVNFFFGALELT									Felis Catus HSD-2
151	FTKAHTTSTGLWGLVNNAGHNEVADAE LSPVATFRSCMEVNFFFGALELT									Homo Sapiens HSD-2
106	FTKAHTTSTGLWGLVNNAGLNDIVADVELSPVATFRKCM EVNFFFGVLELT									Cavia Porcellus HSD-2
151	FTKVHTTSTGLWGLVNNAGQNI FVADAE LCPVATFRKCM EVNFFFGALEMT									Bos Taurus HSD-2
151	ITKAHTASTGLWGLVNNAGLNIVVADVELSPVATFRKCM EVNFFFGALELT									Mus Musculus HSD-2
151	ITKAHTASTGLWGLVNNAGLNIVVADVELSPVATFRKCM EVNFFFGALELT									Rattus Norvegicus HSD-2
KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIAL LMDTFSC EL										Majority
130	KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIA L LMDTFSC EL									Felis Catus HSD-2
201	KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIA L LMDTFSC EL									Homo Sapiens HSD-2
156	KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIA L LMDTFSC EL									Cavia Porcellus HSD-2
201	KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIA L LMDTFSC EL									Bos Taurus HSD-2
201	KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIA L LMDTFSC EL									Mus Musculus HSD-2
201	KGLLPLLRSSRGRIVTVGSPA GDMPPYPCLAAYGT SKAAIA L LMDTFSC EL									Rattus Norvegicus HSD-2
LPWGVKVSII IQPGCFKTESVTNVGLWEK RKQLLLANLPRELLQAYGEDYI										Majority
180	LPWGVKVSII IQPGCFKTESVTNVGLWEK RKQLLLASLPRELLQ									Felis Catus HSD-2
151	LPWGVKVSII IQPGCFKTESVTNVGLWEK RKQLLLANLPRELLQAYGKDYI									Homo Sapiens HSD-2
206	IPWGVKVSII IQPGCFKTESVTNVGLWEK RKQLLLANLPRELLQAYGEDYI									Cavia Porcellus HSD-2
251	LPWGVKVSII IQPACFKTESVKDVHQWEERKQLLLATLPQELLQAYGEDYI									Bos Taurus HSD-2
251	LPWGVKVSII IKPGCFKTD AVTNVNLWEK RKQLLLANIPRELLQAYGEDYI									Mus Musculus HSD-2
251	LPWGVKVSII IQPGCFKTEAVTNVNLWEK RKQLLLANLPRELLQAYGEDYI									Rattus Norvegicus HSD-2
EHLHGQFLNSLRMALPDLSPVVD AITDALLAAQPRRRYYYPGRGLGLMYFI										Majority
222	EHLHGQFLNSLRMALPDLSPVVD AITDALLAAQPRRRYYYPGRGLGLMYFI									Felis Catus HSD-2
256	EHLHGQFLNSLRMALPDLSPVVD AITDALLAAHPRRRYYYPGRGVGLMYFI									Homo Sapiens HSD-2
301	EHLHGQFLNSLRMALPDLSPVVD AITDALLAAQPRRRYYYPGRGVGLMYFI									Cavia Porcellus HSD-2
301	EHLHGQFLNSLRMALPDLSPVVD AITDALLAAQPRRRYYYPGRGVGLMYFI									Bos Taurus HSD-2
301	EHLHGQFLNSLRMALPDLSPVVD AITDALLAAQPRRRYYYPGRGVGLMYFI									Mus Musculus HSD-2
301	EHLHGQFLNSLRMALPDLSPVVD AITDALLAAQPRRRYYYPGRGVGLMYFI									Rattus Norvegicus HSD-2

Fig. 4. Amino acid sequence of a part of feline 11 $\beta$ -HSD2 in comparison with other species. The underlined amino acids indicate the five regions of the enzyme. The most prominent regions are region A, the co-factor binding pocket (82–115), and region D, the active site of the enzyme (232–249). The amino acids underlined separately are mutation sites known to cause AME in humans. The mutations are: deletion of LE, substitution of R187C, R208C, R213C, R279C, D244N, L250R, L251S, R186C, P227L, R337C and R208H. The circumscribed parts are the conserved parts within the regions of the SCAD superfamily.

compares to the characteristics of 11 $\beta$ -HSD1. It is an NADP(H)-dependent enzyme with bidirectional activity. The affinity for cortisol and cortisone was equal for both the dehydrogenase and reductase activity. The enzyme velocity of the dehydrogenase reaction is higher than the reductase reaction over the entire substrate concentration range as illustrated in Fig. 1. This indicates predominant

dehydrogenase activity of this enzyme in the kidney, enabling the feline kidney effectively to convert cortisol into cortisone over a large substrate concentration range.

The presence of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 in the feline kidney was confirmed by RT-PCR. In the kidney of the rat [28], mouse [32] and guinea pig [6], activities of both 11 $\beta$ -HSDs are clearly established. In contrast, in humans only

activity of 11 $\beta$ -HSD2 has been found [19,20,27,47]. However, in these experiments, only kidney cortex and not medulla was used. In the medulla of the human kidney, low mRNA expression and activity of 11 $\beta$ -HSD1 was found by Whorwood [36]. By selectively cutting out kidney cortex, this feature is missed in many experiments. We detected mRNA of 11 $\beta$ -HSD1 and -2 in the feline kidney cortex and in the medulla. In these experiments, cortex and medulla were separated visually. Preliminary activity studies in microsomes of kidney medulla did not show any activity, whereas in the kidney cortex we could clearly establish activity of both 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 (data not shown). This suggests that both 11 $\beta$ -HSD types are expressed in the feline kidney, but in particular the cortex constitutes high enough expression to establish measurable enzyme activity.

In the feline liver, an NADPH-dependent reductase was found, with a  $K_m$  in the micromolar range indicating 11 $\beta$ -HSD1 [48,49]. Corresponding with the results of the enzyme kinetic data, RT-PCR confirmed the identity as 11 $\beta$ -HSD1. In most species, 11 $\beta$ -HSD1 has bidirectional enzyme activity with predominantly reductase activity in the liver. However, our data could only demonstrate unidirectional reduction. Because of slow reaction velocity together with the extreme low substrate affinity, no dehydrogenase activity was detectable. According to previous data, we found bidirectional activity of this enzyme in the kidney [6,28,32]. Hence the enzyme kinetic parameters of 11 $\beta$ -HSD1 in the liver and kidney show remarkable differences. The  $K_m$  of 11 $\beta$ -HSD1 was smaller in the kidney than in the liver (see Tables 1 and 2). Variance in the  $K_m$  values of 11 $\beta$ -HSD in different organs has also been described in guinea pig [6] and rat [50] indicating tissue-specific features. As the cDNA fragment in the liver was 100% homologous with the 11 $\beta$ -HSD1 fragment found in the kidney, this strongly suggests that the NADP(H)-dependent form in the kidney is based on the same gene product as the NADP(H)-dependent form in the liver.

Sequence analysis of the fragments confirmed highly conserved parts. For each enzyme, the active site and the cofactor binding pocket are considered to be the best conserved regions [20]. Comparison of the cofactor binding pocket of feline 11 $\beta$ -HSD2 with that of other species showed the highest homology with humans. Only one amino acid (alanine 104) was different from the human enzyme (serine 104), whereas in the other species alanine or threonine was found at this site. Besides this specific amino acid locus, all other species differ in two to five amino acids from the binding pocket of human 11 $\beta$ -HSD2, indicating the cat to be the most closely related species to man.

A very important amino acid near the cofactor binding pocket in humans is glutamate115 because deletion of this locus in humans is associated with signs of Apparent Mineralocorticoid Excess Syndrome (AMES) and hypertension [51]. At this position glutamate is also found in the cat, while in the other species aspartate115 is found. Other

frequent mutation sites described causing AMES in humans are substitution of R187C, R208C, R213C, R279C, D244N, L250R, L251S, R186C, P227L, R337C and R208H [25,51,52]. Another important finding is that cats share with humans the non-mutated amino acids at all these sites. Thus, for the diagnosis of AMES and related hypertension, all these amino acids are of pivotal interest.

At the active site of 11 $\beta$ -HSD2 considerable species differences exist at position 239 (valine in man, methionine in cats, leucine in cows, isoleucine in guinea pigs, mice and rats). This is remarkable because this locus is considered to be highly conserved in the SCAD superfamily, thus suggesting this amino acid to be the same within one enzyme [20]. Besides this difference, the cat has one other distinct amino acid (serine 234), but this locus is not within the highly conserved part of the SCAD superfamily. As at the cofactor binding pocket, the guinea pig has again the highest diversity at the active site. Besides the cofactor binding pocket (region A) and the active site (region D), there are three other regions (B, C and E) highly conserved in the SCAD superfamily. Their physiological function, however, remains to be elucidated. The cat showed 100% homology with humans in these three regions. Region B is identical in the species described, in region C the guinea pigs and cows have the exceptional amino acids in highly conserved parts, and region E showed differences with humans in all described species except cats.

In the other enzyme, 11 $\beta$ -HSD1, the cofactor binding pocket of the cat exhibits a 100% homology to humans, although we were unable to determine the first three amino acids. At the active site, the cat showed the highest homology with man. The other species differ in one or more amino acids from the cats and humans [5,29,32,53,54]. At the active site of 11 $\beta$ -HSD1, cats and cows have methionine at position 199, while in man and rats this is lysine, in mice and sheep this is threonine, and in guinea pig this is serine, indicating a number of possible variations at this site. The two absolute conserved consensus sites for *N*-glycosylation are present in the cat, and most other species, but not in the guinea pig and sheep where one of these two sites is absent [5,52].

In conclusion, in the kidney of the cat, the predominant activity of 11 $\beta$ -HSD is the conversion of cortisol into cortisone, emphasizing the high capacity of the kidney for inactivation of cortisol. Two types of 11 $\beta$ -HSD are commonly found in the kidney: 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2. With regard to the pathogenesis of hypertension, 11 $\beta$ -HSD2 is the most important type. Feline 11 $\beta$ -HSD2 has similar enzyme kinetic parameters as human 11 $\beta$ -HSD2 and the amino acid sequence of the most important parts of this enzyme shares approximately 90% homology with human 11 $\beta$ -HSD2. Thus, out of all other species investigated as yet, the 11 $\beta$ -HSD enzymes in the cat resemble most closely the human form. By performing enzyme kinetic studies together with sequencing of the most important regions (and possible mutation sites) of the enzymes, we created the tools to investigate the presence



of symptoms of AMES in cats with hypertension. This is of importance not only for feline patients, but it also implies that the cat may serve as a model species for studies directed to the pathogenesis and treatment of AMES and hypertension in humans.

## Acknowledgements

I would like to thank Jaap van Hellemond for the interesting discussions, and Roel Maas for technical assistance.

## References

- [1] S.H. van Uum, et al., The role of 11 beta-hydroxysteroid dehydrogenase in the pathogenesis of hypertension, *Cardiovasc. Res.* 38 (1) (1998) 16–24.
- [2] M.L. Ricketts, et al., Immunohistochemical localization of type 1 11beta-hydroxysteroid dehydrogenase in human tissues, *J. Clin. Endocrinol. Metab.* 83 (4) (1998) 1325–1335.
- [3] C.M. Monder, Heterogeneity of 11 beta-hydroxysteroid dehydrogenase in rat tissues, *J. Steroid Biochem. Mol. Biol.* 40 (4–6) (1991) 533–536.
- [4] E. Maser, B. Volker, J. Friebertshauser, 11 Beta-hydroxysteroid dehydrogenase type 1 from human liver: dimerization and enzyme cooperativity support its postulated role as glucocorticoid reductase, *Biochemistry* 41 (7) (2002) 2459–2465.
- [5] X. Pu, K. Yang, Guinea pig 11beta-hydroxysteroid dehydrogenase type 1: primary structure and catalytic properties, *Steroids* 65 (3) (2000) 148–156.
- [6] M. Quinkler, et al., Evidence for isoforms of 11 beta-hydroxysteroid dehydrogenase in the liver and kidney of the guinea pig, *J. Endocrinol.* 153 (2) (1997) 291–298.
- [7] C. Monder, V. Lakshmi, Y. Miroff, Kinetic studies on rat liver 11 beta-hydroxysteroid dehydrogenase, *Biochim. Biophys. Acta* 1115 (1) (1991) 23–29.
- [8] K.H. Nwe, et al., Differential regulation of the oxidative 11beta-hydroxysteroid dehydrogenase activity in testis and liver, *Steroids* 65 (1) (2000) 40–45.
- [9] K. Yang, M.M. Yu, Evidence for distinct isoforms of 11 beta-hydroxysteroid dehydrogenase in the ovine liver and kidney, *J. Steroid Biochem. Mol. Biol.* 49 (2–3) (1994) 245–250.
- [10] T.J.M. Cole, Cloning of the mouse 11 beta-hydroxysteroid dehydrogenase type 2 gene: tissue specific expression and localization in distal convoluted tubules and collecting ducts of the kidney, *Endocrinology* 136 (10) (1995) 4693–4696.
- [11] E. Maser, G.M. Bannenberg, 11 Beta-hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds, *Biochem. Pharmacol.* 47 (10) (1994) 1805–1812.
- [12] T.Q. Cai, et al., Induction of 11beta-hydroxysteroid dehydrogenase type 1 but not -2 in human aortic smooth muscle cells by inflammatory stimuli, *J. Steroid Biochem. Mol. Biol.* 77 (2–3) (2001) 117–122.
- [13] N.M. Morton, et al., Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice, *J. Biol. Chem.* 276 (44) (2001) 41293–41300.
- [14] C.B. Whorwood, et al., Regulation of glucocorticoid receptor alpha and beta isoforms and type I 11beta-hydroxysteroid dehydrogenase expression in human skeletal muscle cells: a key role in the pathogenesis of insulin resistance? *J. Clin. Endocrinol. Metab.* 86 (5) (2001) 2296–2308.
- [15] P.M. Stewart, B.A. Murry, J.I. Mason, Human kidney 11 beta-hydroxysteroid dehydrogenase is a high affinity nicotinamide adenine dinucleotide-dependent enzyme and differs from the cloned type I isoform, *J. Clin. Endocrinol. Metab.* 79 (2) (1994) 480–484.
- [16] Z. Kyzossev, P.D. Walker, W.B. Reeves, Immunolocalization of NAD-dependent 11 beta-hydroxysteroid dehydrogenase in human kidney and colon, *Kidney Int.* 49 (1) (1996) 271–281.
- [17] M. Shimojo, et al., Immunodetection of 11 beta-hydroxysteroid dehydrogenase type 2 in human mineralocorticoid target tissues: evidence for nuclear localization, *Endocrinology* 138 (3) (1997) 1305–1311.
- [18] Z. Krozowski, et al., Immunohistochemical localization of the 11 beta-hydroxysteroid dehydrogenase type II enzyme in human kidney and placenta, *J. Clin. Endocrinol. Metab.* 80 (7) (1995) 2203–2209.
- [19] S. Kataoka, et al., 11Beta-hydroxysteroid dehydrogenase type 2 is expressed in the human kidney glomerulus, *J. Clin. Endocrinol. Metab.* 87 (2) (2002) 877–882.
- [20] A.L. Albiston, et al., Cloning and tissue distribution of the human 11 beta-hydroxysteroid dehydrogenase type 2 enzyme, *Mol. Cell. Endocrinol.* 105 (2) (1994) R11–R17.
- [21] G. Hirasawa, et al., Colocalization of 11 beta-hydroxysteroid dehydrogenase type II and mineralocorticoid receptor in human epithelia, *J. Clin. Endocrinol. Metab.* 82 (11) (1997) 3859–3863.
- [22] P. Ferrari, Z. Krozowski, Role of the 11beta-hydroxysteroid dehydrogenase type 2 in blood pressure regulation, *Kidney Int.* 57 (4) (2000) 1374–1381.
- [23] M. Shams, et al., 11Beta-hydroxysteroid dehydrogenase type 2 in human pregnancy and reduced expression in intrauterine growth restriction, *Hum. Reprod.* 13 (4) (1998) 799–804.
- [24] Z. Krozowski, et al., The type I and type II 11beta-hydroxysteroid dehydrogenase enzymes, *J. Steroid Biochem. Mol. Biol.* 69 (1–6) (1999) 391–401.
- [25] A. Li, et al., Molecular basis for hypertension in the “type II variant” of apparent mineralocorticoid excess, *Am. J. Hum. Genet.* 63 (2) (1998) 370–379.
- [26] Y. Kotelevtsev, et al., Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2, *J. Clin. Invest.* 103 (5) (1999) 683–689.
- [27] S. Diederich, et al., Metabolism of synthetic corticosteroids by 11 beta-hydroxysteroid-dehydrogenases in man, *Steroids* 63 (5–6) (1998) 271–277.
- [28] J.J. Hermans, et al., Comparison of 11 beta-hydroxysteroid dehydrogenase in spontaneously hypertensive and Wistar–Kyoto rats, *Steroids* 60 (11) (1995) 773–779.
- [29] A.K. Agarwal, et al., Cloning and expression of rat cDNA encoding corticosteroid 11 beta-dehydrogenase, *J. Biol. Chem.* 264 (32) (1989) 18939–18943.
- [30] R. Franco Saenz, et al., Regulation of the genes for 11beta-hydroxysteroid dehydrogenase type 1 and type 2 in the kidney of the Dahl rat, *J. Hypertens.* 17 (8) (1999) 1089–1093.
- [31] Y.J. Liu, Y. Nakagawa, T.M. Ohzeki, Gene expression of 11beta-hydroxysteroid dehydrogenase type 1 and type 2 in the kidneys of insulin-dependent diabetic rats, *Hypertension* 31 (3) (1998) 885–889.
- [32] V. Rajan, et al., Cloning, sequencing and tissue-distribution of mouse 11 beta-hydroxysteroid dehydrogenase-1 cDNA, *J. Steroid Biochem. Mol. Biol.* 52 (2) (1995) 141–147.
- [33] J. Condon, et al., Ontogeny and sexual dimorphic expression of mouse type 2 11beta-hydroxysteroid dehydrogenase, *Mol. Cell. Endocrinol.* 127 (2) (1997) 121–128.
- [34] J. McKinnell, et al., Regulation of 11beta-hydroxysteroid dehydrogenase enzymes by dietary sodium in the rat, *Endocr. Res.* 26 (1) (2000) 81–95.
- [35] E. Maser, G.M. Bannenberg, The purification of 11 beta-hydroxysteroid dehydrogenase from mouse liver microsomes, *J. Steroid Biochem. Mol. Biol.* 48 (2–3) (1994) 257–263.
- [36] C.B. Whorwood, et al., Detection of human 11 beta-hydroxysteroid dehydrogenase isoforms using reverse-transcriptase-polymerase chain reaction and localization of the type 2 isoform to renal collecting ducts, *Mol. Cell. Endocrinol.* 110 (1–2) (1995) R7–R12.

- [37] S. Kong, et al., Absence of type 1 11 $\beta$ -hydroxysteroid dehydrogenase enzyme in koala liver, *Comp. Biochem. Physiol., Part C Pharmacol. Toxicol.* 131 (1) (2002) 39–50.
- [38] M.Y. Zhou, et al., Cloning, expression, and tissue distribution of the rat nicotinamide adenine dinucleotide-dependent 11  $\beta$ -hydroxysteroid dehydrogenase, *Endocrinology* 136 (9) (1995) 3729–3734.
- [39] F. Maggio, et al., Ocular lesions associated with systemic hypertension in cats: 69 cases (1985–1998), *J. Am. Vet. Med. Assoc.* 217 (5) (2000) 695–702.
- [40] N. Ruiz-Opazo, L.V. Lopez, V.L. Herrera, The dual AngII/AVP receptor gene N119S/C163R variant exhibits sodium-induced dysfunction and cosegregates with salt-sensitive hypertension in the Dahl salt-sensitive hypertensive rat model, *Mol. Med.* 8 (1) (2002) 24–32.
- [41] F. Amenta, M.A. Di Tullio, D. Tomassoni, Arterial hypertension and brain damage—evidence from animal models (review), *Clin. Exp. Hypertens.* 25 (6) (2003) 359–380.
- [42] F. Sugiyama, K. Yagami, B. Paigen, Mouse models of blood pressure regulation and hypertension, *Curr. Hypertens. Rep.* 3 (1) (2001) 41–48.
- [43] V. Lakshmi, C.M. Monder, Purification and characterization of the corticosteroid 11  $\beta$ -dehydrogenase component of the rat liver 11  $\beta$ -hydroxysteroid dehydrogenase complex, *Endocrinology* 123 (5) (1988) 2390–2398.
- [44] A.A. Rutten, et al., Interlaboratory comparison of total cytochrome P-450 and protein determinations in rat liver microsomes. Reinvestigation of assay conditions, *Arch. Toxicol.* 61 (1) (1987) 27–33.
- [45] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.R. Jmolec, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [46] F.W.M. Perrella, EZ-FIT: a practical curve-fitting microcomputer program for the analysis of enzyme kinetic data on IBM-PC compatible computers, *Anal. Biochem.* 174 (2) (1988) 437–447.
- [47] S. Diederich, et al., In the search for specific inhibitors of human 11 $\beta$ -hydroxysteroid-dehydrogenases (11 $\beta$ -HSDs): chenodeoxycholic acid selectively inhibits 11 $\beta$ -HSD-I, *Eur. J. Endocrinol.* 142 (2) (2000) 200–207.
- [48] P.M. Jamieson, et al., 11  $\beta$ -hydroxysteroid dehydrogenase type 1 is a predominant 11  $\beta$ -reductase in the intact perfused rat liver, *J. Endocrinol.* 165 (3) (2000) 685–692.
- [49] Z. Krozowski, The 11 $\beta$ -hydroxysteroid dehydrogenases: functions and physiological effects, *Mol. Cell. Endocrinol.* 151 (1–2) (1999) 121–127.
- [50] R.S. Ge, et al., Identification of a kinetically distinct activity of 11 $\beta$ -hydroxysteroid dehydrogenase in rat Leydig cells, *Endocrinology* 138 (6) (1997) 2435–2442.
- [51] A. Odermatt, et al., A mutation in the cofactor-binding domain of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 associated with mineralocorticoid hypertension, *J. Clin. Endocrinol. Metab.* 86 (3) (2001) 1247–1252.
- [52] R.C. Wilson, et al., A genetic defect resulting in mild low-renin hypertension, *Proc. Natl. Acad. Sci. U. S. A.* 95 (17) (1998) 10200–10205.
- [53] U.C. Oppermann, K.J. Netter, E.M. Maser, Cloning and primary structure of murine 11  $\beta$ -hydroxysteroid dehydrogenase/microsomal carbonyl reductase, *Eur. J. Biochem.* 227 (1–2) (1995) 202–208.
- [54] G.M. Tannin, et al., The human gene for 11  $\beta$ -hydroxysteroid dehydrogenase. Structure, tissue distribution, and chromosomal localization, *J. Biol. Chem.* 266 (25) (1991) 16653–16658.